Customer application note | 001063

HPLC

Parallel analysis of drug product for assay and related substances determination

Authors

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Keywords

Metformin hydrochloride, 1-cyanoguanidine, assay, related substances, impurity, Vanquish Duo, Dual LC, HPLC, UHPLC, productivity, throughput

Application benefits

- Increased productivity on dual-channel (U)HPLC system compared to single-channel system
- Optimal bench space utilization with Thermo Scientific™ Vanquish™ Duo UHPLC system
- Efficient use of laboratory staff time
- Improved cost of ownership in terms of maintenance contracts after instrument warranty is over

Goal

To demonstrate faster product release by simultaneous analyses of active pharmaceutical ingredient and its related impurities using a Vanquish Duo UHPLC system

Introduction

Pharmaceutical laboratories performing routine analysis of drug products are often tasked with the processing of a large number of samples. The number of simultaneously running HPLC systems is a key factor that dictates the turnaround time for sample processing. Therefore, labs often require numerous systems, and consequently large laboratories, to fulfill their task. The Vanquish offers an ideal solution to significantly increase productivity.[1] The system provides two separate flow paths with the footprint of a single (U)HPLC system, doubling throughput while efficiently utilizing laboratory

bench space (Figure 1). Consequently, more applications can be performed with the same number of systems in one laboratory. A sample can be completely characterized for drug content and impurities with only one sample vial, one HPLC system, and one set of solvents at one time. Other aspects are a reduced number of laboratory staff and minimizing human error. For example, for eluent preparations, eluents only need to be prepared once if they are shared between both flow paths in the same system. On the other hand, a single user can only operate a limited number of instruments at the same time, even if the bench space is available. The Vanquish Duo UHPLC system therefore increases an analyst´s ability to run two methods simultaneously by operating only one instrument.

Approaches that strongly benefit from the Dual LC are:

- Two completely independent applications
- Two applications that use the same eluents but with different gradient methods and/or different target analyte concentration
- One method performed simultaneously on both flow paths to double throughput
- Method development and validation (e.g., robustness testing by testing columns from different batches)
- Mass balance study for the same set of samples of an API, where an optical (e.g., UV) and a non-optical (e.g., CAD) detector are required

This application note describes a comprehensive analysis of a drug substance, running the same chromatographic method but requiring different run times and target analyte concentrations for the analysis of an assay and impurity determination of metformin hydrochloride in tablets. With conventional (U)HPLC systems, this would either require two different systems or sequential analysis (Figure 2).

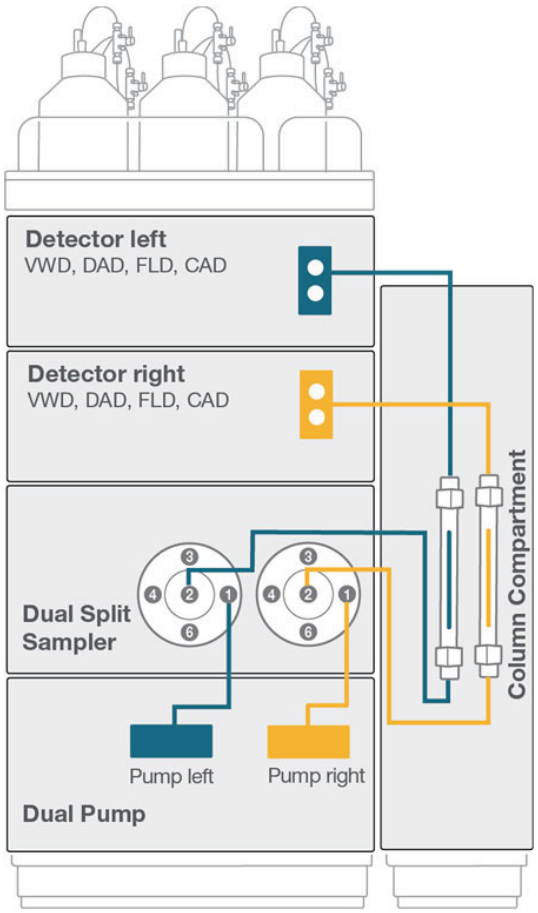

Figure 1. Scheme of the Vanquish Duo UHPLC system with two completely independent flow paths and two detectors. In case of the requirement of two different column temperatures, a second column compartment can be added.

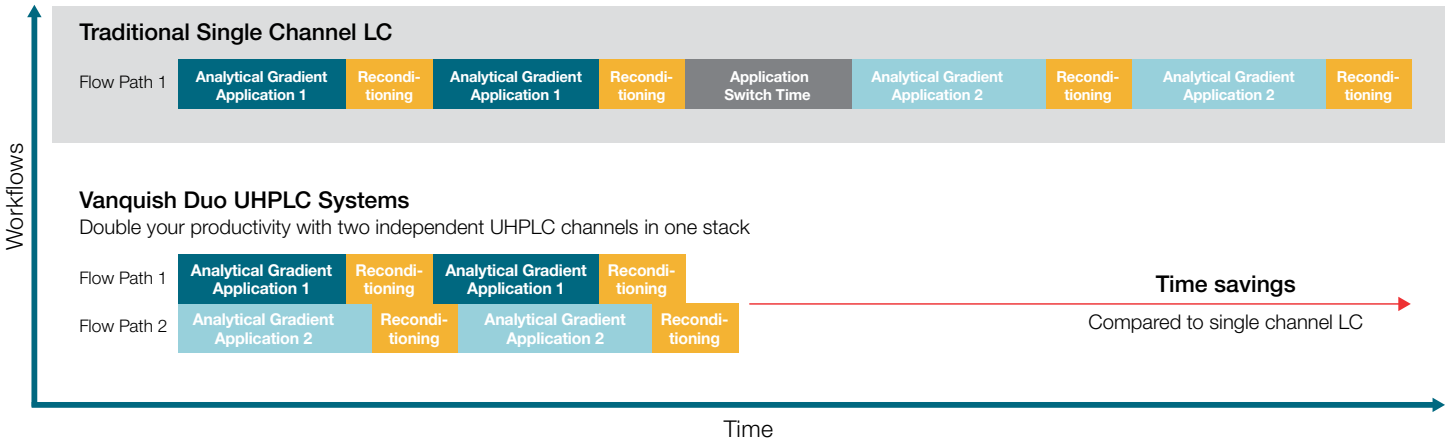

Figure 2. Comparison between a single channel (top) and the dual channel LC (bottom) when performing two different applications

Experimental

Chemicals

Name	Part number
ASTM Type I water for HPLC system	
Acetonitrile, Merck, HPLC grade	DC2DF72431
Sodium heptane sulfonate, Merck, AR grade	DJ0D70228
Sodium chloride, Merck, AR grade	MK0M700074
Ortho-phosphoric acid 85%, Merck, AR grade	DA2D720009

Sample handling

Name	
Rotary shaker, Biotechnics India	
Vials, Borosil™, Merck	
Cap and septum, Borosil™, Merck	

Instrumentation

Module	Part number
Vanquish Flex Duo UHPLC system consisting of:	
System Base Vanquish Duo UHPLC system	VF-S02-A-02
Vanquish Dual Pump F	VF-P32-A
Vanquish Dual Split Sampler FT	VF-A40-A-02
Vanquish Column Compartment H	VH-C10-A-02
Vanquish Diode Array Detector HL (2x)	VH-D10-A
Standard LightPipe flow cell, biocompatible (2x)	6083.0100B

Preparation of solvents

Preparation of ortho-phosphoric acid solution for pH adjustment

5.4 g of ortho-phosphoric acid (85%) were accurately weighed and transferred to a 1,000 mL volumetric flask, made up to volume with water, and mixed until completely dissolved.

Preparation of buffer solution

500 mg each of sodium heptane sulfonate and sodium chloride were dissolved into a 1 L volumetric flask, 900 mL of water was added, and the solution was mixed. The pH was adjusted to pH 3.85 ± 0.05 with ortho-phosphoric acid solution and diluted with water to 1,000 mL.

Preparation of mobile phase

The filtered and degassed mixture of the above prepared buffer solution and acetonitrile were used in the ratio 90/10 (v/v).

Preparation of diluent solution

A mixture of water and acetonitrile was prepared in the ratio 70/30 (v/v).

Preparation of assay standards and samples

Preparation of blank solution

Diluent was used as a blank solution.

Preparation of standard stock solution (1.25 mg/mL)

125.0 mg of metformin hydrochloride were weighed and transferred accurately into a 100 mL volumetric flask. 30 mL of diluent solution were added, and the solution was sonicated for 2 minutes to dissolve the metformin hydrochloride. The solution was then diluted to volume with diluent solution.

Preparation of standard solution (125 µg/mL)

2.0 mL of standard stock solution were pipetted and diluted to 20 mL with diluent and mixed thoroughly.

Preparation of sample solution (125 µg/mL)

Tablets (no fewer than 20) were finely powdered. A powder equivalent to about 125 mg of metformin hydrochloride was transferred to a previously dried 100 mL volumetric flask. 30 mL of acetonitrile were added, and the solution sonicated for 10 minutes. 20 mL of water were added, and the solution was shaken for 45 minutes.

Then, 20 mL of water were added, and the solution was sonicated for an additional 20 minutes. The solution was allowed to stand for a few minutes to cool to room temperature before being made up to volume with water and then filtered through a 0.45 µm nylon filter.

2.0 mL of this filtered test solution was further diluted to 20 mL with diluent.

Preparation of related substances (RS) standards and samples

Preparation of sample solution (1.25 mg/mL)

Tablets (no fewer than 20) were finely powdered. A powder equivalent to about 125 mg of metformin hydrochloride was transferred to a previously dried 100 mL volumetric flask. 50 mL of diluent were added, and the solution was sonicated for 5 minutes. The solution was allowed to stand for a few minutes to cool to room temperature before being made up to volume with water and then filtered through a 0.45 µm nylon filter.

Preparation of placebo solution

A placebo powder equivalent to about 125 mg of metformin hydrochloride was transferred into a previously dried 100 mL volumetric flask. 50 mL of diluent were added, and the solution was sonicated for 5 minutes. The solution was allowed to stand for a few minutes to cool to room temperature before being made up to volume with diluent and mixed thoroughly. The solution was filtered through a 0.45 µm nylon filter.

Preparation of metformin hydrochloride standard stock solution (125 µg/mL)

125.0 mg of metformin hydrochloride were weighed and accurately transferred into a 100 mL volumetric flask. 30 mL of diluent were added, and the solution was sonicated for 2 minutes to dissolve the metformin hydrochloride completely before being made up to volume with diluent and mixed thoroughly.

2 mL of standard stock solution were pipetted out, diluted to 20 mL with diluent, and mixed thoroughly.

Preparation of 1-cyanoguanidine standard stock solution (25 µg/mL)

About 5.0 mg of 1-cyanoguanidine were weighed and transferred into a 200 mL volumetric flask. About 140 mL of diluent were added and the solution was sonicated for 5 minutes to dissolve the 1-cyanoguanidine. The solution was allowed to stand for a few minutes to cool to room temperature, diluted to volume with diluent, and mixed thoroughly.

Preparation of standard solution

1.0 mL of metformin hydrochloride standard stock solution and 1.0 mL of 1-cyanoguanidine standard stock solution were pipetted into a 100 mL volumetric flask and diluted to volume with diluent.

Preparation of sensitivity solution (0.25 µg/mL)

0.2 mL of metformin hydrochloride standard stock solution were diluted to 100 mL with diluent.

Table 1. Chromatographic conditions

Column	Phenomenex HyperClone™ BDS C18 (250 x 4.6 mm; 5 µm), P/N ACE-121-2546
Mobile phase	90/10 buffer/acetonitrile (v/v)
Isocratic run time (Assay)	15 min
Isocratic run time (RS)	30 min
Flow rate	1.0 mL/min
Column temperature	30 °C with active pre-heater at 30 °C (still air)
Sampler temperature	25 °C
Injection volume	10 µL (Assay) 5 µL (RS)
UV detector parameters	Detection at 218 nm
	Data collection rate: 10 Hz
	Response time: 0.05 s

Chromatography data system

Thermo Scientific™ Chromeleon™ 7.2.10 Chromatography Data System (CDS) was used for data acquisition and processing.

Results and discussion

The in-house methods for the determination of metformin hydrochloride and related substances in tablets were performed on a Vanquish Flex Duo, a modern UHPLC instrument with two fully independent flow paths. This allows the two methods to be run in parallel rather than sequentially as would be the case for a single flow path system. Furthermore, a single analyst performed the analysis on one system. Analysis for assay and related substances was conducted, requiring a C18 column of 250 x 4.6 mm and 5 µm particle size. The HyperClone BDS C18 column fulfilled all required system suitability test (SST) criteria. This UHPLC instrument can easily be coupled with this conventional HPLC column resulting in a backpressure of about 240 bar. The result showed excellent retention time and peak area reproducibility.

Assay

The sequence started with two blank injections to determine whether there were any interferences for the target analyte region in the chromatogram, with the result of no interferences being observed. The standard solution (125 µg/mL) was then injected consecutively six times to evaluate tailing factor, theoretical plate number, and relative standard deviation of peak area (RSD peak area). Figure 3A shows six overlaid injections of the standard solution (125 µg/mL). Tailing factor, theoretical plate number, and RSD peak area on metformin were 1.61, 16877, and 0.05%, respectively, which are well below the SST requirements (tailing factor ≤2.0, theoretical plate number ≥2,000 and RSD peak area ≤2.0%). In addition, an excellent relative standard deviation of retention time (RSD RT) of 0.01% could be achieved.

Three different samples (125 µg/mL) were prepared twice and injected once. All three samples were found to be in the specification range of 95–105% (97.1% for sample 1, 97.4% for sample 2, and 98.2% for sample 3) with metformin hydrochloride content in the tablets.

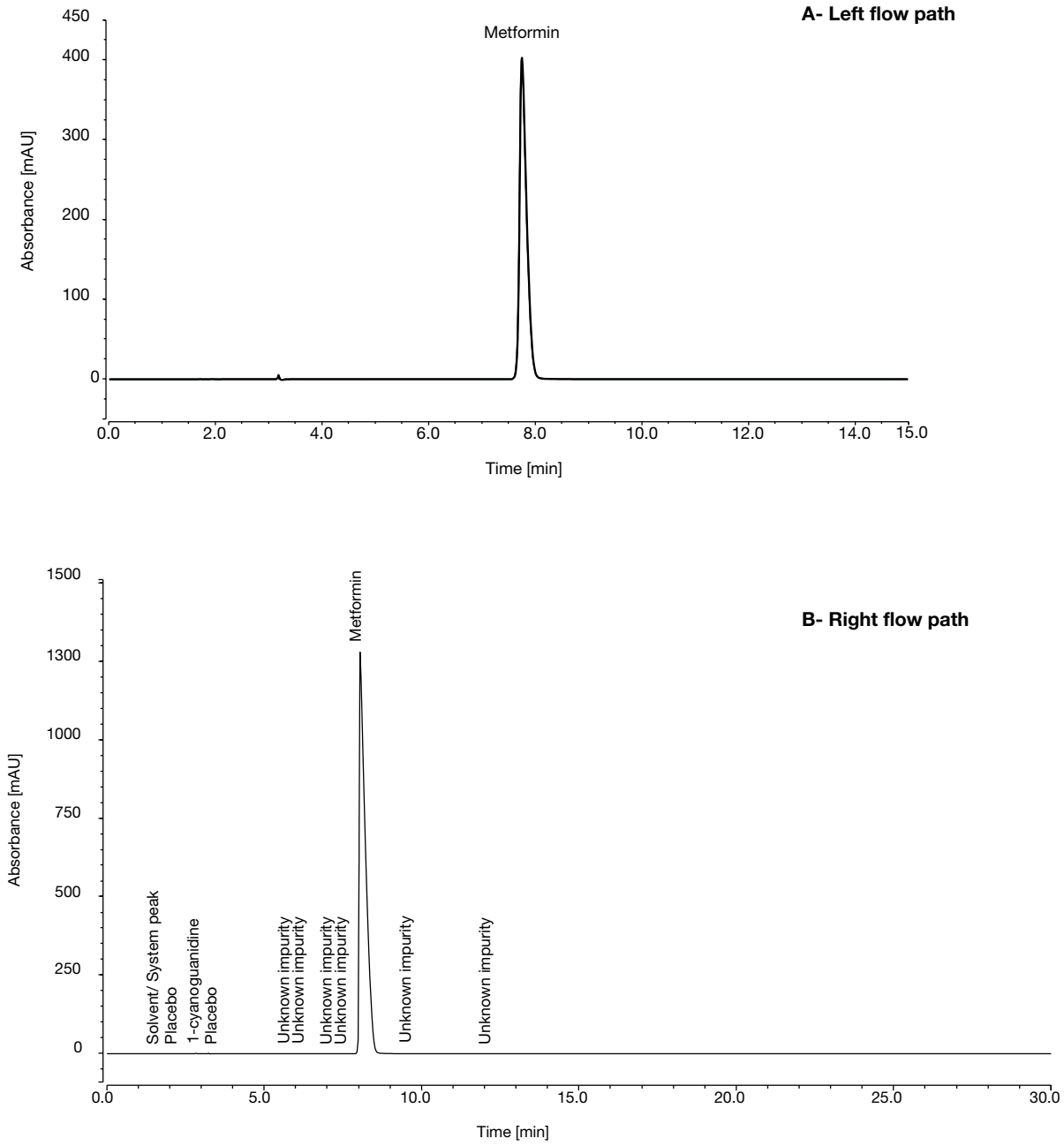

Figure 3. (A) (left flow path) – overlaid chromatograms of six replicate injections of the standard solution (assay) containing 125 µg/mL metformin hydrochloride and (B) (right flow path) – chromatogram of sample solution (RS) containing 1.25 mg/mL metformin hydrochloride. Both on HyperClone BDS C18 (250 x 4.6 mm, 5 µm) column with buffer solution/acetonitrile (90/10 v/v), injection volume 10 µL (assay) and 5 µL (RS), column temperature 30 °C, UV wavelength 218 nm.

Related substances

As for the assay method, two blank injections were performed at the beginning of the sequence and no interferences were detected. The sequence continued with a single injection of the placebo solution to determine any matrix peaks that may be present, which are then not considered in the evaluation of related substances in the samples. Then, the sensitivity solution (0.02% API) was injected once to determine the minimum peak area to be considered for sample data evaluation.

To assess SST criteria, six consecutive standard solution injections were performed. The requirement is defined as follows: RSD peak area ≤10% and tailing factor ≤2.0.

Table 2 summarizes the limit of detection (LOD) and limit of quantification (LOQ) of the method as well as the obtained results for SST.

Two different samples (1.25 mg/mL) were prepared and injected once. The chromatogram of one sample is shown in Figure 3B. As can be seen in Table 3, all impurity levels obtained were below the acceptance criteria.

Conclusion

By performing the analyses on the Vanquish Duo UHPLC system, increased productivity was achieved, resulting in faster product release and saving bench space and laboratory staff time. Although assay and related substances tests can be run separately, preference is given to running these two tests on the same instrument as the materials used are often the same for both methods. Therefore, human error and out-of-specification results are reduced when an analyst only has to prepare solvents and samples once.

- Vanquish Duo UHPLC system enabled parallel execution of assay and related substances analysis for metformin hydrochloride in tablets in one instrument.
- Tablet samples for drug content determination all met the criteria of 95–105%.
- Related impurities in the samples were found below the acceptance limits.

Reference

1. Thermo Fisher Scientific BR72623: Brochure for Vanquish Duo UHPLC Systems. https://assets.thermofisher.com/TFS-Assets/CMD/brochures/br-72623-lc-vanquish-duo-uhplc-br72623-en.pdf

Table 2. LOD and LOQ values for API (metformin) and known impurity (1-cyanoguanidine) and obtained SST results

Compound	LOD	LOQ	SST RSD peak area	SST Tailing factor
Metformin	0.02% (w/w)	0.04% (w/w)	1.31	1.02
1-cyanoguanidine	0.004% (w/w)	0.008% (w/w)	0.35	1.05

Table 3. Acceptance criteria on related substances and obtained results from two different samples

Compound	Acceptance criteria Not more than [%]	Sample 1 Calculated amount	Sample 2 Calculated amount
Metformin	-	-	-
1-cyanoguanidine	0.02	0.012	0.012
Any single unknown impurity	0.1	< LOQ	< LOQ
Total impurities	0.2	0.012	0.012

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